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HORMONE REPLACEMENT AND THE HYPOGLYCAEMIA OF COMBINED TOTAL PANCREATECTOMY AND PARTIAL HEPATECTOMY IN THE RAT

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SUMMARY.—Rats subjected to combined total pancreatectomy and partial hepatectomy became hypoglycaemic some hours later, glucagon administration did not prevent this and insulin accelerated the lowering of the blood sugar.

WHILST investigating the effects of pancreatectomy on liver regeneration, it was found that rats subjected to both total pancreatectomy and partial hepatectomy became profoundly hypoglycaemic (and often completely aglycaemic) some hours after this double operation (Sutton and Taghizadeh, 1968). Although there is a close association between the liver, the pancreas and carbohydrate metabolism, this drop in blood glucose levels could not be explained on the data available. It is known that the liver is the only organ in the body capable of maintaining a normal blood glucose concentration in the fasting state, whilst the pancreas secretes both insulin and glucagon. In the classical experiments of Mann and Magath (1922) total hepatectomy was shown to produce fatal hypoglycaemia in dogs, but in our experiments only two-thirds of the liver was removed, leaving more than the critical 20 per cent of the hepatic mass which is needed to prevent hypoglycaemia (Ross, 1968). We also found that rats undergoing partial hepatectomy alone, did not become hypoglycaemic. The other animals subjected to pancreatectomy became progressively more hyperglycaemic (*i.e.* diabetic) as was expected and served as controls. Because it was possible that the hypoglycaemia was the result of changes in the levels of the pancreatic hormones, the effects of administering glucagon and insulin under these experimental conditions were studied.

MATERIALS AND METHODS

Adult male white Wistar rats, weighing between 140–200 g. were used throughout these experiments. The animals were fully fed on the standard laboratory diet of M.R.C. 41 b cubes, up to the times of their operations, but not given food post-operatively (thereby avoiding differences in food intake between the various groups), though drinking water was always freely available. Total pancreatectomy was performed by a slight modification of Scow's (1957) technique in which 99·5 per cent of the organ is removed. Partial hepatectomy was the classical Higgins and Anderson (1931) operation, taking away two-thirds of the organ. Sham operations in which an abdominal laparotomy was performed, with manipulation of the liver and pancreas were also made. All the operations were performed under open ether anaesthesia, between 10 a.m. and 2 p.m., to avoid diurnal variations. The aortic blood glucose was measured by a glucose-oxidase method on the AutoAnalyser (Marks and Lloyd, 1963), a technique appropriately adapted to give high sensitivity at hypoglycaemic levels above 10 mg. per 100 ml. The hepatic glycogen content was estimated by the micromethod of Kemp and Kits van Heijningen (1954). Glucagon (for injection U.S.P.) manufactured by Eli Lilly, U.S.A., was dissolved in the diluting solution supplied immediately prior to its intraperitoneal injection. Insulin zinc suspension B.P. ("Lente insulin", Burroughs Wellcome, London) was also given by i.p. injection.

RESULTS

Clinical effects.—The operation combining total pancreatectomy with partial hepatectomy takes from 30–50 min. Following this the rats make a good initial recovery, rapidly regaining consciousness, clean themselves, drink and walk around their cages for the next few hours. Then from about 4 hr post-operatively onwards they become much less active and start to shake violently from side to side whilst becoming progressively weaker. Some of them pass gradually into coma and die quietly, while others show powerful extensor spasms of their limbs and back (" convulsions ") and jump about with irregular, uncoordinated jerky movements, suddenly ceasing to breathe and dying rapidly. Either of these clinical states may be associated with pulmonary oedema, as can be judged by the wetness of their circumoral fur. The overall clinical picture is similar both to insulin induced hypoglycaemia in the rat (Korec, 1967) and to the fatal hypoglycaemia which follows total hepatectomy in the dog (Mann and Magath, 1922), and could be prevented by maintaining the rats on 10 per cent glucose solution in place of their drinking water. By contrast rats subjected to pancreatectomy never exhibit these features, their post-operative behaviour being that of increasing diabetic ketosis which leads to their eventual death some 48 hr or so later. Animals undergoing a partial hepatectomy remain very well during the entire post-operative period.

Blood glucose and liver glycogen levels.—As we showed previously (Sutton and Taghizadeh, 1968), rats subjected to both total pancreatectomy and partial hepatectomy, and not given food post-operatively, become fatally hypoglycaemic (often with virtually no detectable blood glucose) some hours after this double procedure, at a time when only very small amounts of glycogen remain in their livers. Table I shows the effects of killing the various groups of rats at set post-operative times, and demonstrates how the hypoglycaemia starts to develop from 4–6 hr later.

TABLE I.—*Mean Blood Glucose (mg./100 ml.) and Hepatic Glycogen (per cent) ± Standard Error (with Numbers of Animals in Parentheses) at 4 and 6 hr After Operation, in 65 Rats*

Time	Pancreatectomy with partial hepatectomy	Pancreatectomy alone	Partial hepatectomy alone	Laparotomy alone
4 hr				
Blood glucose	81 ±14·3 (6)	198·5±19 (6)	81·5 ±4·1 (6)	99·6±0·8 (6)
Liver glycogen	2·2± 0·56 (6)	4·5± 0·71 (6)	0·31±0·02 (6)	3·2±0·34 (6)
6 hr				
Blood glucose	28·8± 7·7 (19)	160 ±18 (6)	80·7 ±8·5 (9)	85·1±4·7 (7)
Liver glycogen	1·0± 0·2 (19)	1·3± 0·43 (6)	0·44±0·15 (9)	1·5±0·25 (7)

In another experiment the animals subjected to both operations were killed only when they were judged to be severely hypoglycaemic as shown in Table II.

Except for the first rat which died of unknown causes before the critical hours had elapsed, all the animals undergoing the double procedure became aglycaemic from 6–9 hr later. However, the animals in the 2 control groups were still alive 24 hr later, when they were killed and the experiment terminated.

TABLE II.—*Blood Glucose in mg./100 ml. and Hepatic Glycogen (per cent) in 18 Rats*

Rat no.	Operation	Clinical state at death	Hours after operation	Blood glucose	Hepatic glycogen
1	Pancreatectomy + Partial Hepatectomy	Died (cause unknown)	5	97	2·7
2	,, ,, ,,	Convulsions, killed	6	<10	0·3
3	,, ,, ,,	Convulsions, killed	7	<10	0·3
4	,, ,, ,,	Unresponsive, killed	8	<10	0·2
5	,, ,, ,,	Unresponsive, killed	8	<10	0·4
6	,, ,, ,,	Unresponsive, killed	9	<10	0·4
7–12	Pancreatectomy alone	Alive but unwell, killed	24	344±15·6*	2·03±0·43*
13–18	Partial hepatectomy alone	Alive and very well, killed	24	50±2·7*	0·23±0·05*

* Mean ± Standard Error.

The effects of glucagon administration.—Initially the effect of glucagon on normal, fully fed rats was studied. Table III shows the response to the hormone, a rather mild and rapid hyperglycaemia similar to that found by other workers using this animal (Korec, 1967).

TABLE III.—*Blood Glucose in mg./100 ml. (Mean of 2 Results) in 12 Rats Given 0·2 mg. Glucagon i.p.*

Time after glucagon	Blood glucose
0	102
10 min.	121
20 ,,	124
30 ,,	112
60 ,,	112
2 hr	82
6 ,,	98

Then 2 groups, each of 6 rats, were subjected to total pancreatectomy combined with partial hepatectomy, and one group given 0·2 mg. glucagon per rat 4 hr post-operatively. All the animals were then killed at 6 hr post-operatively, the results are shown in Table IV.

TABLE IV.—*Blood Glucose in mg./100 ml. in 12 Rats, 6 hr After Total Pancreatectomy Combined with Partial Hepatectomy*

+ Glucagon	No extra treatment
<10	<10
<10	<10
<10	<10
28	55
59	83
99	100

There is a wide scatter in the blood glucose levels, but half of the rats in each group had become aglycaemic. It was not known why the others had failed to develop hypoglycaemia, but it seemed possible that it was merely a question of

time, and that had the experiment been allowed to continue all of the animals would have shown very low blood glucose levels. Accordingly another experiment was performed in which the rats were killed only when they showed obvious clinical evidence of hypoglycaemia. Eight rats were subjected to the double operation, and given 0·2 mg. glucagon immediately the operation was completed, followed by another 0·2 mg. 4 hr post-operatively. The results of this experiment are given in Table V.

TABLE V.—*Total Pancreatectomy with Partial Hepatectomy in* 8 *Rats Given Glucagon Post-operatively*

Rat no.	Clinical state at death	Time after operation (hr)	Blood glucose in mg./100 ml.
1	Convulsions, died	3	< 10
2	Unresponsive, killed	6	< 10
3	,, ,,	7	< 10
4	Found dying	7	< 10
5	Unresponsive, killed	7	21
6	,, ,,	8	10
7	,, ,,	8	68
8	,, ,,	9	13

Only one animal failed to develop profound hypoglycaemia, and again this might have been due to killing the rat too soon, on an erroneous clinical impression.

The effects of insulin administration.—In this experiment 4 groups of rats were given 2 units each of insulin, by i.p. injection, immediately after their various operations were completed. They were then observed for up to 6 hr when the experiment was terminated. Table VI gives the incidence and timing of the fatal hypoglycaemia which appeared amongst the groups.

TABLE VI.—*Incidence and Timing of Fatal Hypoglycaemia in* 25 *Rats Each Given* 2 *Units Insulin Post-operatively*

	Pancreatectomy with partial hepatectomy	Pancreatectomy alone	Partial hepatectomy alone	Laparotomy alone
Number of animals	6	6	7	6
Number dead by 6 hr of hypoglycaemia (blood glucose < 10 mg./100 ml.)	6	6	4	1
Mean time of death (hr) post-operatively	2·6 (range 2·0–3·7)	3·4 (range 2·0–4·0)	—	—

Thus all animals undergoing pancreatectomy (with or without partial hepatectomy) were extremely sensitive to the hypoglycaemic effects of insulin in the immediate post-operative period.

DISCUSSION

These results extend our original observations that rats subjected to both total pancreatectomy and partial hepatectomy become fatally hypoglycaemic some hours later, by showing that large doses of glucagon fail to maintain normal blood sugar levels, whereas the administration of insulin accelerates the onset of hypoglycaemia. The production of hypoglycaemia by this double operation was not originally anticipated, since it is well known that a two-thirds partial

hepatectomy does not seriously impair glucose homeostasis, and that total pancreatectomy leads to the hyperglycaemia typical of experimental diabetes mellitus. Why then does the performance of both operations together so drastically lower the blood glucose?

The role of shock.—Shock is defined as the general response of the body to injury (Stoner, 1961), and any surgical operation must entail a certain amount of shock however minor in amount. Removing all of the pancreas together with two-thirds of the liver is a severe injury, and the possibility that the hypoglycaemia is a reaction to the shock must be considered. However, severe hypoglycaemia is not a known complication of surgical shock as seen after extensive operative procedures. We have attempted to increase the amount of shock in experiments in which up to 3 ml. of blood was withdrawn from the jugular veins of rats, which were then partially hepatectomised. Although this is at least 3 times as much blood as would be lost during the double operation, yet hypoglycaemia did not develop in these animals. Nevertheless, Stoner (1958) has found hypoglycaemia in rats severely shocked by total ischaemia of both hind limbs for 4 hr, followed by release of the clamps. In his experiments 85 per cent of the animals die of shock some 13 hr later, but during the first 3–6 hr there is a rise in blood sugar at a time when some of the other parameters of the animal's general condition (temperature and total oxygen consumption) are falling. It is only after this first phase that the animals enter the period of irreversible shock, and then the blood glucose falls to very low levels. Stoner (1961) observes that this latter phase of irreversible shock " might be better called just dying ", and regards the low blood sugar levels as due to a continuous decline in body function in which tissue anoxia from failure of oxygen transport leads to death. He also points out that giving these animals glucose only makes them worse, since it is not utilised by the tissues but accumulates in the blood stream where it exerts a deleterious osmotic effect. In our experiments it is the pancreatectomy which would be expected to cause the most shock, because the operative technique is a rather long and laborious blunt dissection removing all of the portions of the pancreas from the adjacent blood vessels and viscera. By contrast, the partial hepatectomy takes only a few extra min., in which the narrow pedicle separating the 2 major lobes from the rest of the organ is quickly ligated and divided with a minimum of tissue damage. Nevertheless, the post-operative blood sugars of the pancreatectomised rats have always been higher than normal, and never hypoglycaemic. For this reason, and also because the administration of glucose can prevent the hypoglycaemia occurring in our experiments, it seems very unlikely that shock following the operation is the cause of the profound lowering of the blood sugar levels.

The effect of glucagon replacement.—In some birds and reptiles glucagon is known to be an essential hormone, and pancreatectomy leads to hypoglycaemia which can be prevented by the administration of glucagon. Such an effect has never been found in mammals (Marks and Rose, 1965). Nevertheless in our experiments it seemed possible that the additional loss of two-thirds of the liver might unmask an acute deficiency of the hormone; but the giving of large amounts of glucagon failed to maintain normal blood sugar levels. The glucagon used was the same preparation and administered by the same (i.p.) route as in the experiments of Penhose, Houssay and Lujan (1965) in which glucagon replacement in lizards prevented the hypoglycaemia following total pancreatectomy. These negative results are in keeping with current concepts of glucose homeostasis

which shed considerable doubt about the status of mammalian glucagon as a major hyperglycaemic hormone. In a recent review, Marks and Samols (1968) suggest that the physiological role of glucagon is that of a local hormone regulating the secretion of insulin, and that its ability to stimulate glycogenolysis is a pharmacological effect produced by giving it in excessively large amounts.

The effects of insulin.—Since the basic phenomenon under investigation was severe hypoglycaemia, it was regarded as likely that the administration of insulin in these experiments would worsen the situation by further lowering of the blood sugar. This was, in fact, the effect of giving insulin to rats subjected to both total pancreatectomy combined with partial hepatectomy. It was also observed that rats undergoing total pancreatectomy were more insulin sensitive than controls, this was also found by Scow (1957) who noted that pancreatectomised rats may be very insulin sensitive in the immediate post-operative period unless given food. Further work is in progress to see if there is any relationship between the level of plasma insulin and the onset of hypoglycaemia.

The role of the liver.—The other aspect of the problem is the part played by the liver in these experiments, especially since pancreatectomy by itself (with a normal amount of hepatic tissue) resulted in hyperglycaemia and did not cause any lowering of the blood sugar. The timing of the hypoglycaemia, occurring some hours after the double operation, suggested that it developed after the hepatic reserves of glycogen had been exhausted, and this was confirmed by the low levels of liver glycogen found at death (Table II). Further work is needed to establish whether the hypoglycaemia is caused by (*a*) decreased hepatic production of glucose or (*b*) increased tissue consumption of glucose.

Conclusions.—The aim of these experiments was an attempt to find the cause of the hypoglycaemia which follows the removal of all of the pancreas, with two-thirds of the liver, in the rat. For the reasons stated above this is not thought to be a simple (non-specific) consequence of the surgical shock of the 2 operations. Because partial hepatectomy by itself did not result in hypoglycaemia it appeared possible that the loss of some pancreatic factor or factors was responsible for the effect. However, from the data presented here it is clear that acute glucagon deficiency did not produce the lowered blood sugar because the administration of large amounts of this hormone did not prevent it. The cause of the hypoglycaemia therefore remains unknown.

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